Developmental Origins of Increased Nuchal Translucency

Burger, N.B.

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Chapter 1

General introduction and outline of the thesis

INTRODUCTION

The nuchal translucency (NT) is a subcutaneous collection of fluid in the posterior neck region of a fetus[1]. It can be visualized by ultrasound at 10-14 weeks of gestation and disappears in the majority of the fetuses after 14 weeks of gestation[2]. An increased NT is defined as a measurement of the translucent area in the neck region above the 95th percentile for the gestational age[3]. Ultrasound measurement of NT is an important part of first-trimester screening for trisomy 21, trisomy 18 and trisomy 13[4,5]. Increased NT is also associated with monosomy X[6]. In euploid fetuses an increased NT identifies fetuses at risk for cardiac defects[7-13], other structural malformations[10,12], rare genetic syndromes and skeletal dysplasias[10-12]. Nevertheless, the majority of euploid fetuses with increased NT have a healthy, uncomplicated outcome[14].

Although NT measurement is used as part of first-trimester screening, a pathophysiological explanation for increased NT is lacking. Several mechanisms have been suggested, but they fail to explain both the regional and temporary character of nuchal edema, as well as the wide spectrum of fetal anomalies associated with increased NT.

Initially, it was believed that increased NT origins from cardiac failure, either due to myocardial dysfunction or secondary to a cardiac defect[15]. This hypothesis has been based on the high frequency of cardiac defects in euploid and aneuploid fetuses with increased NT[7-13,16-21] and the fact that the risk of a cardiac defect increases with enlargement in NT in euploid fetuses[17]. But not all fetuses with increased NT have a cardiac defect. Furthermore, the most common cardiac defect in fetuses with increased NT is a ventricular septal defect. This type of cardiac defect does not cause cardiac failure in fetal life. In fact, the majority of cardiac defects do not compromise fetal hemodynamics[22]. If cardiac failure causes increased NT, then specific types of cardiac defects that can result in fetal hemodynamic alterations – such as tricuspid valve insufficiency or stenosis – should be overrepresented in fetuses with increased NT. Enlarged NT is, however, not related to a specific type of cardiac defect, but is related to any type of cardiac defect[17,22-24]. Besides, fetal cardiac failure is usually progressive towards fetal death and is not a temporary phenomenon.

Cardiac failure has also been suggested to cause increased NT because of the finding of abnormal ductus venosus flow velocity waveforms in fetuses with increased NT[15,25-28]. It was suggested that cardiac failure causes both increased NT and abnormal ductal flow velocity waveforms. But intra-cardiac flow velocities do not differ between euploid and aneuploid fetuses with normal or enlarged NT, irrespective of cardiac anatomy[29]. The fact that abnormal ductal flow velocity waveforms are also observed in euploid fetuses with normal NT, showing both normal and abnormal cardiac anatomy[30,31], and in aneuploid fetuses with increased NT and normal cardiac anatomy[32] do not support the theory of cardiac failure as an explanation for increased NT.

If cardiac failure causes increased NT, then fetal findings of cardiac decompensation, such as pleural and pericardial effusion, ascites and cardiomegaly, would be expected. These findings are, however, usually not observed in fetuses with increased NT. Cardiac failure can neither

explain the local and temporary fluid accumulation in the neck region. In conclusion, the contention of cardiac failure as explanation for increased NT is commonly presumed but is based on assumptions and is supported by neither evidence nor pathophysiology.

Previous studies showed abnormally enlarged jugular lymphatic sacs in the neck of fetuses with increased NT, together with the finding that increased NT is in fact mesenchymal edema[33-35]. It was suggested that a disturbance in lymphatic development caused an increased NT. A longitudinal ultrasound study in fetuses with increased NT showed a temporal relationship between the development of NT and the volume of the enlarged jugular lymphatic sacs, in which increased NT preceded the jugular lymphatic sac expansion[36]. Lymphatic development starts in the neck by the formation of the jugular lymphatic sacs[37]. Lymphatic endothelial cells bud and migrate from the cardinal veins as single cells or clusters and subsequently form the jugular lymphatic sacs[38]. The jugular lymphatic sacs then remodel into lymph nodes after 10 weeks of gestation[39]. The development of the lymphatic system is completed by the ingrowth of the right thoracic duct into the left jugular lymphatic sac, thereby forming the main drainage site of lymphatic fluid into the venous systemic circulation[39,40]. A delay or disturbance in lymphatic development could explain the temporary and local fluid accumulation in the nuchal region. Immunohistochemical studies showed a diminished expression of lymphatic markers Prox-1 and Podoplanin and an increased expression of blood vessel markers, such as Neuropilin-1, Smooth Muscle Actin (SMA) and Vascular Endothelial Growth Factor (VEGF)-A in the jugular lymphatic sacs in aneuploid mouse embryos and human fetuses with nuchal edema[41-43]. Abnormal (lymphatic) endothelial development could explain the finding of enlarged jugular lymphatic sacs and nuchal edema.

Morphological studies in trisomy 16 mouse embryos with nuchal edema demonstrated an abnormal development and altered positioning of cranial nerves, which are consistently located adjacent to the jugular lymphatic sacs[44]. It was suggested that abnormal neuronal development could disturb lymphatic endothelial differentiation of the jugular lymphatic sacs[44]. Cranial nerves are derived from the neural crest and develop in the same time period in the late first trimester as nuchal edema[45]. Disturbances in neural crest cell migration or differentiation cause cardiac defects[46-48], craniofacial abnormalities[47] and skeletal malformations[49], which are anomalies that are often found in fetuses with increased NT.

In summary, a lymphatic developmental disorder seems to be involved in the etiology of increased NT. Disturbed endothelial and neuronal development seem to be prominent denominators in the pathophysiology of increased NT.

AIM AND OUTLINE OF THE THESIS

This thesis describes the translational approach to explain the clinical finding of fetal increased NT. The aim of this thesis was to study the relationship between increased NT, lymphatic development, cardiovascular development and the involvement of nerves to provide more insight into the developmental background of increased NT.

In **chapter 2** we have identified genes involved in normal cardiac and lymphatic development. Loss of function of these genes causes both cardiac and lymphatic abnormalities in mouse embryos with nuchal edema. This provides insight into the underlying genetic mechanisms that can explain the high incidence of cardiac defects in fetuses with increased NT.

In **chapter 3** the involvement of cranial nerve progenitor cells, the neural crest cells, has been investigated in lymphatic development. Retinoic acid is synthesized in nerves and is essential for neural crest cell and lymphatic development. The involvement of retinoic acid at specific stages of lymphatic differentiation and in nuchal edema has been examined.

In **chapter 4** the theory of cardiac failure as explanation for increased NT has been investigated. The nuchal region, jugular lymphatic sacs and cardiac anatomy have been studied in various mutant mouse models with lymphatic and cardiac defects with and without the presence of nuchal edema.

Altered hemodynamics, reflected by abnormal ductus venosus flow velocity waveforms, has been suggested to be involved in the development of increased NT. **Chapter 5** describes the use of a uniform anatomical definition for the ductus venosus. In **chapter 6** the systematic morphological development of the ductus venosus and the presence of a ductus venosus sphincter have been studied in wild-type mouse and human embryos. In **chapter 7** we have investigated whether a local alteration in ductus venosus morphology causes abnormal ductus venosus flow velocity waveforms in several mutant mouse models with lymphatic and cardiac defects with and without the presence of nuchal edema. In **chapter 8** the origin of abnormal ductus venosus flow velocity waveforms and its relationship with increased NT and cardiac defects has been further explored in human fetuses. Postmortem examination of the ductus venosus, heart and jugular lymphatic sacs has been performed in human fetuses with normal and abnormal ductus venosus flow velocity waveforms, showing normal and increased NT. This thesis ends with a general discussion and future perspectives in **chapter 9**. A summary of the presented data is given in both English and Dutch in **chapter 10**.

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